6th ICNTC 2020 Special Issue

J. Indian Chem. Soc.
Vol. 97, October(C) 2020

Stabilization and Harvesting of Hesperetin Microrods from the Hesperetin Stabilised Via Plantacare 2000 Solution by Using Template-Assisted Particle Infiltration Method

Yesim KARAHAN[1], Marcel POURASGHAR[2], Marc SCHNEIDER[2], Cornelia M. KECK[3], Dolunay SAKAR DASDAN[1]

[1]Yıldız Technical University, Faculty of Art and Science, Chemistry Department, 34210, İstanbul, Turkey

[2]Saarland University,Department of Pharmacy, Biopharmaceutics and Pharmaceutical Technology, 66123, Saarbrücken, Germany

[3]Marburg University,Pharmaceutical Technology and Biopharmacy, 35037,Marburg, Germany

*E-mail: dsakar@yildiz.edu.tr

Manuscript Received online 10/30/2020, Accepted 11/17/2020

Recent studies have shown that the particle properties of drugs play a major role for drug delivery systems. One of the important factors in pulmonary drug delivery is the particle shape, and studies in the literature show that cylindrical rods have the highest acquisition and binding efficiency. Hesperetin has antioxidant, anti-carcinogenic, anti-hypertensive, anti-atherogenic anti-inflammatory etc. effects and its solubility in water is very low. Considering all this information Hesperetin microrods for use in pulmonary drug delivery were tried to be obtained from Hesperetin dispersed with Plantacare2000 by applying template-assisted method with particle infiltration approach which has three steps: infiltration, stabilization and harvest. In this study, microrods inside the pores which are obtained by Hesperetin-Plantacare 2000 solution infiltrating into pores of track-etched polycarbonate membranes, were stabilized by using different polymers and harvested.

Keywords: Hesperetin, Plantacare2000, microrods, Template-assisted method, Polycarbonate Track-Etched Membranes, Agarose

Introduction

Hesperetin [(2S)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydrochromen-4-one] (Fig.1) is a flavanone and an aglycone form of hesperidin found in citrus fruits[1] Hesperidin taken with diet is converted to hesperetin via deglycosylation by intestinal bacteria before absorption. It shows pharmacological properties such as anti-inflammatory, anti-carcinogenic, anti-hypertensive, anti-atherogenic, antioxidant, antiallergic[2-4] Also, it has blood lipid lowering effects[5] and the ability to penetrate to blood-brain barrier[6]

The lung has large surface area and short distance between the epithelium and blood vessels. These properties allow rapid local and systemic effect by directly administering the drug to the target area. Inhalational administration of drugs provide many advantages, such as in lower and upper respiratory tract diseases, sufficient dose of active substance can be transported directly to the effected area, minimizing systemic side effects regular clinical response, removal of

biological barriers such as first pass effect observed with oral use, they can be used at much lower doses for the same therapeutic effect compared to the dose of active substance taken orally, removing of conditions that reduce patient compliance (bad taste, pain etc.), suitable for delivering many substances from small molecules to large peptide molecules, large surface area and high permeability membrane structure advantages such as low enzymatic activity[7-16]. In pulmonary drug delivery, the particle shape is an important factor for uptake of the carrier systems by phagocytes. A high particle length diameter ratio delays uptake into macrophages. This results in higher bio-adjustment and an effective therapeutic effect. The particle acquisition and binding efficiency of the cylindrical rods is highest[17-19].

In order to obtain microrods, template- assisted particle infiltration method is an useful and effective way[20-23]. This method relies on the infiltration of the nanoparticle containing solution into the template to make it shape. After this, by using different material, their shape is stabilized. Finding a solvent that does not dissolve the material to be infiltrated but dissolves the membrane is the importing task. In general, Track-etched membranes are used as the template. Because; their physicochemical properties and being both inorganic acid and organic polymers provide advantages in finding suitable solvents. They have parallel, straight and uniform sized pores which allows obtaining a high aspect ratio and large amounts of rods. Active substances are filled into cylindrical material. The obtained microrods are stabilized with various polymers. Thus, their shapes are conserved.

Agarose is a polysaccharide usually extracted from some red seaweed, a linear polymer consisting of the repeating agarobiosis unit of the disaccharide composed of D-galactose and 3,6-anhydro-L-galactopyranose[24]. Agarose forms an uncharged polymer with hydrogen bonds with temperature. Concentration affects gel pore size. Thus, separation properties become adjustable[25]. D-Mannitol has water-soluble property and mucotic effect. By creating an osmotic gradient that facilitates water flow into the airway lumen, it has a modifying effect on the viscoelastic properties associated with sputum in the airway, making it pulmonary useful[26,27]. L-leucine is used as an auxiliary agent in stabilization with mannitol because it prevents agglomeration due to its surfactant property[28].

At the stage of collecting microrods from stabilized membranes, the polycarbonate membrane is dissolved in a suitable solvent (THF, N-methyl-2-prolidone, chloroform, methylene chloride) and removed from the media.

Experimental

5% Hesperetin-Plantacare2000 (180nm) and 5%Hesperetin-Plantacare2000 (400nm) were obtained from Pharmaceutical Technology and Biopharmacy of Philips-University Marburg. D-Mannitol, L-Leucine, Sodiumhydroxide, and 25 mm diameter Whatman Nuclepore Track-Etched Polycarbonate Membranes (5µm and 0.1µm pore sized) were obtained from Sigma Aldrich. Agarose Sea Prep. were obtained from Lonza.

Legato 210 Kdscientific syringe pump was used for infiltration, Heraeus Multifuge X3 centrifuge for centrifugation and Carl Zeiss EVO HD15 Scanning Electron Microscope (SEM) was used for characterization.

In order to determine the most suitable concentration of Hesperetin-

Plantacare2000 (180nm) 0.01; 0.05; 0.1;0.2 and 0.3 mg/ml Hesperetin-Plantacare2000 solutions were preparedby using 5% Hesperetin-Plantacare2000 (180nm) and milliq water. 3ml of each solution was taken and injected to the 5µm pore sized polycarbonate membrane. The solutions prepared in different concentrations were put into the syringe of the programmable syringe pump in 3 ml, respectively. The syringe with a holder attached to its tip was placed on the pump. The solution was infiltrated into the membranes by applying the 2 * 3ml program (injecting 3 ml solution twice). The program is in four stages and includes infiltration at 1ml / min in the first step, 15 minutes stand in the second step, infiltration at 0.1ml / min in the third step and 10 minutes stand in the last step. The infiltrated membranes were cleaned with a 0.01M NaOH solution -moistened tissue and sampled for SEM. In order to determine the most effective particle size 0.1 mg/ml Hesperetin-Plantacare2000 (400nm) solution was prepared by using 5% Hesperetin-Plantacare2000 (400nm) and milliq water. And 3ml of solution was infiltrated to the 5 µm pore sized membrane by using same programme twice. 0.1 µm pore sized membrane was used as a block membrane. For calculating infiltration efficieny membranes' empty and full pores are counted by using Image-J Win64 Programme. Infiltration efficiency calculated with Eq.1.[29]

$$Infiltration\ Efficency = \frac{full\ pores\ number}{total\ pores\ number} \times 100 \qquad \text{Eq.1}$$

In order to stabilize with Agarose, the mixture obtained by adding 1.5 mg Agarose into distilled water was heated at about 80^{0}C and stirred to dissolve.1.5% (m / V) Agarose solution was prepared. 0.3 ml of 1.5% (m / V) Agarose solution was placed on lamella. Hesperetin infiltrated membrane was placed on the lamella with its shiny surface in contact with the solution. 1 day was waited for drying. In order to stabilize with D-Mannitol ,20% (m / V) Mannitol Solution; 20 mg D-Mannitol and 1mg Leucine were prepared by mixing in water at 50-60 ^{0}C. 0.3 ml of 20% (m / V) Mannitol solution was placed on glass lamella for stabilization of Hesperitin microrods with 20% (m / V) Mannitol solution. Hesperetin infiltrated membrane was placed on the lamella with its shiny surface in contact with the solution. One day was waited for drying.

On account of harvesting of Hesperetin microrods, the membrane stabilized with D-Mannitol and Agarose taken from the lamellas was taken into 2 ml Eppendorfs tubes and dissolved separately. Different solvents were used as the solvent: Chloroform ($CHCl_3$), Benzylether ($C_{14}H_{14}O$), Chloroform-Benzylether Mix (1: 3) and Chloroform + Toluene Mix (1: 2). And by shaking, the membranes were dissolved. To separate the microrods from the mixture that is dissolved with $CHCl_3$; The appropriate centrifuge conditions were investigated with different temperature, time and speed settings: ~690 rcf 20 min 20 ^{0}C, ~690 rcf 30 min 20 ^{0}C, ~2700 rcf 20 min 20^{0}C, ~11000 rcf 20 min 20^{0}C and ~2700 rcf 20 min 10 ^{0}C. It was centrifuged three times using 2ml solvent each time at ~690 rcf, 20 min 20^{0}C settings for other solvents.

Results and discussion

SEM images of membranes infiltrated with Hesperetin-PC2000 (180nm) solution at different concentrations are given in Fig. 1.

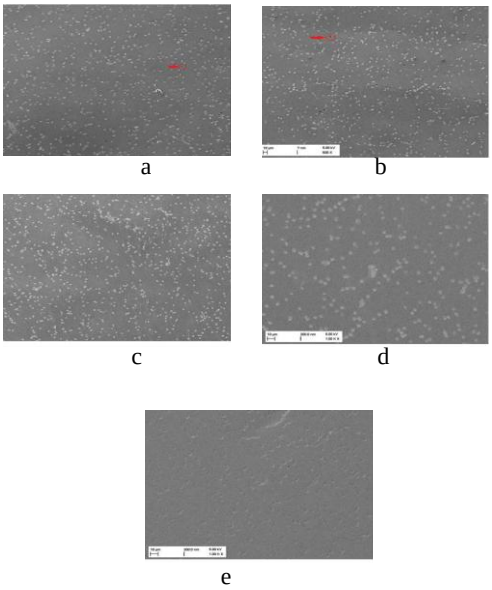


Fig. 1. SEM images of samples infiltrated with a) 0.01 mg/ml b) 0.05mg/ml c) 0.1 mg/ml d) 0.2 mg/ml e) 0.3 mg/ml Hesperetin-Plantacare2000 (180nm) solutions

According to the SEM images, empty pores are present in the infiltration of 0.01mg / ml and 0.05mg / ml Hesperetin solutions, and almost all of the pores are filled in the infiltration of 0.1mg / ml, 0.2mg / ml, 0.3mg / ml Hesperetin solutions (Fig. 1).

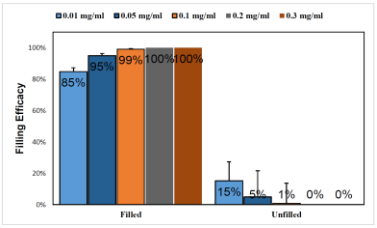


Fig. 2. Infiltration efficiency of 0.01; 0.05; 0.1; 0.2 and 0.3 mg / ml Hesperetin-Plantacare 2000 (180nm) solutions

The infiltration efficiency of the samples infiltrated with Hesperetin-PC2000 (180nm) solution at different concentrations is given in Fig.2.

Considering the efficiency, it was decided that the optimal concentration of Hesperetin-PC 2000 (180nm) solution's infiltration was 0.1 mg / ml.

0.1mg/ml Hesperetin-Plantacare2000 (180nm) infiltrated membrane's and 0.1 mg/ml Hesperetin-Plantacare2000 (400nm) infiltrated membrane's SEM images are given in Fig. 3 and their infiltration efficiency graphs are given in Fig. 4.

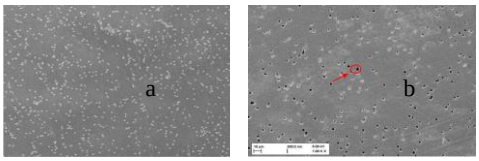


Fig. 3 a) 0.1mg/ml Hesperetin-Plantacare2000 (180nm) 0.1 mg/ml b) Hesperetin-Plantacare2000 (400nm) infiltrated membrane's SEM images

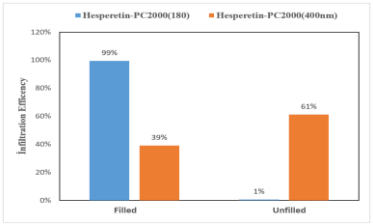


Fig. 4. Infiltration efficiency of 0.1mg/ml Hesperetin-Plantacare2000 (180nm) and Hesperetin-Plantacare2000 (400nm)

As shown at Fig. 3. Hesperetin-Plantacare2000 (400nm) infiltrated membrane has empty pores. When Hesperetin-Plantacare2000(180nm)'s infiltration efficiency is 99%, Hesperetin-Plantacare2000(400nm)' s infiltration efficiency is just 39%(Fig. 4.)[29].

On the harvest step, when chloroform was used as a solvent, sediment could only be obtained with ~11000 rcf 20 min 20 ^{0}C settings.

For other solvents, settings are ˜690 rcf 20 min 20^{0}C. SEM images of harvested microrods by using Agarose as a stabilizer and different solvent are given in Fig.5 and by using D-Mannitol as a stabilizer are given in Fig. 6.

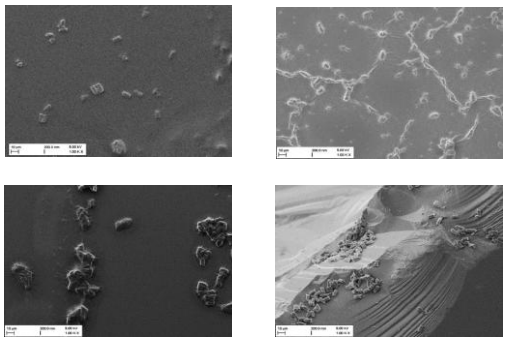
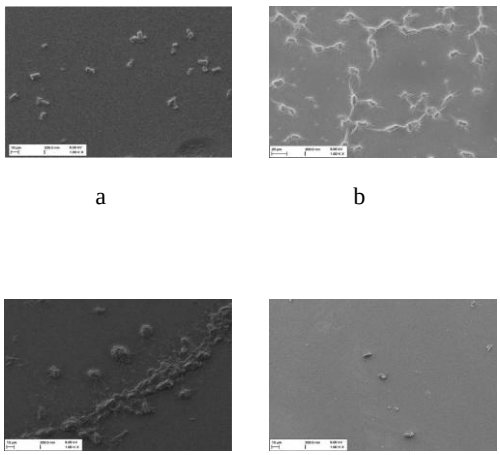


Fig. 5. SEM images of microrods which is stabilized with Agarose and harvested with a) Chloroform b) Benzylether c) Chloroform+Benzylether d) Chloroform+ Toluen



a b

c d

Fig. 6. SEM images of microrods which is stabilized with D-Mannitol and harvested with a) Chloroform b) Benzylether c) Chloroform+Benzylether d) Chloroform+ Toluen

Conclusions

This study shows that Template-Assisted particle infiltration method can be used for preparing Hesperetin microrods. In infiltration step, 0.1 mg/ml is the optimal concentration with 99% efficiency for the most efficient infiltration with the least amount of Hesperetin-Plantacare2000 (180nm). Also, in this study particle size was considered. Hesperetin-Plantacare2000 (400nm) solutions was not effective (39%). It is thought that the increase in particle size increases the space between particles, reducing the interaction of the particles by reducing the contact surface, thus preventing these particles from clinging to each other and settling in the pores. For harvesting, Chloroform+Toluen mixture is the best option as it requires lower centrifugal forces than others. It was observed that there were more microrods stabilized with Agarose than with D-mannitol.

Acknowledgements

This study was carried out by the Higher Education Council (YÖK) with the 100/2000 YÖK Doctorate Scholarship Program; was supported by Yıldız Technical University Erasmus Department with the Erasmus$^+$ Program and by Yıldız Technical University Research Fund (Project number: FDK-2020 3853).

References

1. P. Mouly, E. M. Gaydou and A. Auffray, *J. Chromatogr A.* 1998, **800**,171–179;
2. A. Garg, S. Garg, L. J. Zaneveld and A. K. Singla, *Phytother Res.,* 2001, **15**, 655–669;
3. Y.Z. Cai, Q. Luo, M. Sun and H. Corke, *Life Sci,* 2004, **74**, 2157–2184;
4. N. Lin, T. Sato, Y. Takayama, Y. Mimaki, Y. Sashida, M. Yano and A. Ito, *Biochem Pharmacol,* 2003, **65**, 2065–2071;

5. N.M. Borradaile, K.K. Carroll and E.M. Kurowska, *Lipids*, 1999, **34**, 591;
6. Y. Mitsunaga, H. Takanaga, H. Matsuo, M. Naito, T. Tsuruo, H. Ohtani and Y. Sawada, *Eur. J. Pharmacol,* 2000, **395**, 193-201;
7. N.R. Labiris and M.B. Dolovic, *British Journal of Clinical Pharmacology,* 2003, **56(6)**, 588-599;
8. D.A. Groneberg, C. Witt, U. Wagner, K.F. Chung and A. Fischer, *Respiratory Medicine,* 2003, **97(4)**, 382-387;
9. M. Brzoska, K. Langer, C. Coester, S. Loitsch, T.O. Wagner and C. Mallinckrodt, *Biochemical and Biophysical Research Communications* 2004, **318(2)**, 562-570;
10. M. Jansen, I Darby, T Abribat, P. Dubreuil, E.S. Ferdinandi and J.G Hardy, *International Journal of Pharmaceutics* 2004, **276(1–2),** 75-81;
11. C. Bosquillon, V. Préat and R. Vanbever, *Journal of Controlled Release*, 2004, **96(2)**, 233-244;
12. M. Bivas-Benita, K.E. van Meijgaarden, K.L.M.C. Franken, H.E. Junginger, G. Borchard, T.H.M. Ottenhoff and A. Geluk, *Vaccine*, 2004, **22(13–14)**, 1609-1615;
13. P.N. Reynolds, *Methods in Molecular Biology,* 2004, **246**, 69-89;
14. J.D. Brain, *Diabetes Technology & Therapeutics*, 2007, **9** Suppl 1, 4-15;
15. H. Okamoto, H. Todo, K. Lida and K. Danjo, *Kona,* 2002, **20**, 71-83;
16. K.A. Johnson, *Advanced Drug Delivery Reviews,* 1997, **26(1)**, 3-15;
17. S. Barua, J.W. Yoo, P. Kolhar, A. Wakankar, R.Y. Gokarn and S. Mitragotri, *PNAS,* 2013, **110**, 3270-3275;
18. J.A. Champion and S. Mitragotri, *PNAS* 2005, **103**, 4930–4934;
19. J. Möller, T. Luehmann, T. Hall and V. Vogel, *Nano Lett.* 2012, **12**, 2901–2905;
20. M. Möhwald, S.R. Pinnapireddy, B. Wonnenberg, M. Pourasghar, M. Jurisic, A. Jung, C. Fink-Straube, T. Tschernig, U. Bakowsky and M. Schneider, *Adv Healthc Mater,* 2017, **6**;
21. A. Torge, G. Pavone, M. Jurisic, K. Lima-Engelmann and M. Schneider, *Aerosol Sci Technol*, 2018, **53**, 53-62;
22. D. Kohler, M. Schneider, M. Krüger, C.M. Lehr, H. Möhwald and D. Wang, *Adv Mater,* 2011, **23,** 1376-1379;
23. C. Tscheka, M. Hittinger, C.M. Lehr, N. Schneider-Daum and M. Schneider, *Eur J Pharm Biopharm*, 2015, **95**, 151-155;
24. M.C. Rodríguez, M.C. Matulewicz, M.D. Noseda, D.R.B. Ducatti and P.I. Leonardi, , *Bioresource Technology*, 2009, **100(3)**, 1435-1441;
25. N.C. Stellwagen and E. Stellwagen, *Journal of Chromatography A*, 2009, **1216(10)**, 1917-1929;
26. C.B. Burness and G.M. Keating, *Drugs*, 2012, **72**,1411-1421;
27. N. Barraud, A. Buson, W. Jarolimek and S.A. Rice, *PloS one*, 2013, **8(12),** e84220;
28. P.H. Moghaddam, V. Ramezani, E. Esfandi, A. Vatanara, M. Nabi-Meibodi, M. Darabi, K. Gilani andA.R. Najafabadi, *Powder Technol*,2013, **239**, 478-483;
29. Y. Karahan, M. Pourasghar, M. Schneider, C.M. Keck and D. Sakar- Dasdan, *32. National Chemistry Congress*, 2020, 80;